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Preparation of Auto-induction Terrific Broth for Protein Expression

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Protocol status: In development

We are still developing and optimizing this protocol

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Abstract

The following is the default scale for this protocol for each reagent:

10X Terrific Broth without phosphates: 3 L
 100X Magnesium sulfate (MgSO_4 , 125 mM): 1 L
 100X Ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$, 2.5 M): 1 L
 20X Potassium phosphate (KH_2PO_4 , 960 mM): 1 L
 10X Sodium phosphate (Na_2HPO_4 , 500 mM): 1 L
 100X D-Glucose ($\text{C}_6\text{H}_{12}\text{O}_6$, 1.60 M): 1 L
 10X α -Lactose ($\text{C}_{12}\text{H}_{24}\text{O}_{12}$, 222 mM): 1 L
 50% Glycerol ($\text{C}_3\text{H}_8\text{O}_3$, 6.84 M, 630 g/L): 1 L
 1X Auto-induction Terrific Broth (made fresh from stocks per production run): 1 L

If you change the scale of the protocol through protocol.io's scaling feature when running the protocol, the scaled values are as follows:

10X Terrific Broth without phosphates:  3 L
 100X Magnesium sulfate (MgSO_4 , 125 mM):  1 L
 100X Ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$, 2.5 M):  1 L
 20X Potassium phosphate (KH_2PO_4 , 960 mM):  1 L
 10X Sodium phosphate (Na_2HPO_4 , 500 mM):  1 L
 100X D-Glucose ($\text{C}_6\text{H}_{12}\text{O}_6$, 1.60 M):  1 L
 10X α -Lactose ($\text{C}_{12}\text{H}_{24}\text{O}_{12}$, 222 mM):  1 L
 50% Glycerol ($\text{C}_3\text{H}_8\text{O}_3$, 6.84 M, 630 g/L):  1 L
 1X Auto-induction Terrific Broth (made fresh from stocks per production run):  1 L

The values for all components throughout the protocol should scale accordingly as well. The changes to the suggested container sizes may not make sense, so choose your container appropriately when changing the scale of the protocol. You can see this part of the abstract again by switching to the "Abstract" tab when running the protocol.



Protocol materials

☒ Magnesium sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M1880-500G**

☒ Ammonium sulfate **Santa Cruz Biotechnology Catalog #sc-29085**

☒ Potassium Phosphate, Monobasic **Santa Cruz Biotechnology Catalog #sc-203211A**

☒ α -Lactose monohydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L2643-500G**

☒ D-glucose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #G7021-1KG**

☒ Sodium phosphate dibasic heptahydrate **Santa Cruz Biotechnology Catalog #sc-203402A**

☒ 1000x Trace Metals Mixture. 1000mL, Sterile **Teknova Catalog #T1001**

☒ Yeast Extract **IBI Scientific Catalog #IB49162**

☒ Tryptone **ApexBio Technology Catalog #20-251**



10X Terrific Broth without phosphates and glycerol

2h 55m

- 1 Prepare a  4 L **beaker** to mix broth components. Use a bottle for which you have verified the graduations are reliable. 5m
- 1.1 Using a  2 L **graduated cylinder**, measure  2 L of Milli-Q/deionized water, and pour the water into the beaker.
- 1.2 Place the beaker on a **hot plate** that is set to  80 °C .
- 1.3 Let the water reach at least  70 °C . 10m

- 2 Measure and mix dry components with water to obtain a final volume of  3 L . 45m
 
- 2.1 On a **balance**, measure  720 g of  Yeast Extract **IBI Scientific Catalog #IB49162** in a  1 L **beaker**.
- 2.2 Slowly add the yeast extract powder to the  4 L beaker with water until everything has dissolved. Stir using a reagent spatula as you add the powder to prevent clumping.
- 2.3 On a balance, measure  360 g of  Tryptone **ApexBio Technology Catalog #20-251** in a  1 L **beaker**.
- 2.4 Slowly add the tryptone powder to the  4 L beaker with water until everything has dissolved. Stir using a reagent spatula as you add the powder to prevent clumping.
- 2.5 Top-up the beaker to  3000 mL with Milli-Q/deionized water.
- 2.6 Transfer the media to a  5 L glass media bottle using an appropriately sized **funnel**.



2.7 Close the bottle, leaving the cap slightly ajar, and affix autoclave tape to the cap and bottle.

3 Place the bottle of media in an autoclave pan filled with a 4 cm layer of water, and autoclave the media at 121 °C liquid cycle for 00:55:00 .

55m

4 After autoclaving is complete, let the bottle cool to Room temperature to 40 °C before handling.

40m

Note

Do not tighten the bottle cap while cooling.

5 Under an open flame, make single use aliquots of 100 mL in **sterile** 125 mL **PET bottles**.

10m



Note

Keeping the base 10X Terrific Broth separately aliquoted out in single-use bottles allows for flexibility on how it might be used by different people across the lab (agar plates, smaller plasmid production runs, etc.). It also helps prevent potential contamination. Adjust aliquoting as needed for your lab.

6 On 1.375" x 1.375" thermal labels, label each bottle with the following information:

Base 10X Terrific Broth- No phosphates, no glycerol

120g/L Tryptone

240g/L Yeast extract

Store at room temperature.

Sterile, autoclaved.

Date of manufacture: YYYYMMDD

100X Magnesium sulfate (MgSO₄, 125mM)

15m

7 Prepare a 2 L **beaker** to mix components.



7.1 Using a  1 L **graduated cylinder**, measure  700 mL of Milli-Q/deionized water, and pour the water into the beaker.

7.2 Add a **magnetic stir bar** to the beaker and turn on the **magnetic stir plate**.

8 On a balance, measure  30.8 g

 Magnesium sulfate heptahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #M1880-500G**

8.1 Slowly add the powder to the Milli-Q/deionized water until everything has dissolved.

8.2 Pour the solution back into a  1 L graduated cylinder.

8.3 Top up the volume to  1 L with Milli-Q/deionized water.

8.4 Filter sterilize using a 0.22µm bottle top filter.

9 On a 1.375" x 1.375" thermal label, label the bottle with the following information:

100X Magnesium sulfate (MgSO_4 , 125mM)

30.8g/L Magnesium sulfate heptahydrate

Store at room temperature.

Sterile, 0.22µm filtered.

Date of manufacture: YYYYMMDD

100X Ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$, 2.5M)

15m

10 Prepare a  2 L **beaker** to mix components.

10.1 Using a  1 L **graduated cylinder**, measure  700 mL of Milli-Q/deionized water, and pour the water into the beaker.



10.2 Add a **magnetic stir bar** to the beaker and turn on the **magnetic stir plate**.

11 On a balance, measure  330 g

 Ammonium sulfate **Santa Cruz Biotechnology Catalog #sc-29085** .

11.1 Slowly add the powder to the Milli-Q/deionized water until everything has dissolved.

11.2 Pour the solution back into a  1 L graduated cylinder.

11.3 Top up the volume to  1 L with Milli-Q/deionized water.

11.4 Filter sterilize using a 0.22µm bottle top filter.

12 On a 1.375" x 1.375" thermal label, label the bottle with the following information:

100X Ammonium sulfate ((NH₄)₂SO₄, 2.5M)

330g/L Ammonium sulfate

Store at room temperature.

Sterile, 0.22µm filtered.

Date of manufacture: YYYYMMDD

20X Potassium phosphate (KH₂PO₄, 960mM)

15m

13 Prepare a  2 L **beaker** to mix components.

13.1 Using a  1 L **graduated cylinder**, measure  700 mL of Milli-Q/deionized water, and pour the water into the beaker.

13.2 Add a **magnetic stir bar** to the beaker and turn on the **magnetic stir plate**.



14 On a balance, measure  130 g



Potassium Phosphate, Monobasic **Santa Cruz**
Biotechnology Catalog #sc-203211A

14.1 Slowly add the powder to the Milli-Q/deionized water until everything has dissolved.

14.2 Pour the solution back into a  1 L graduated cylinder.

14.3 Top up the volume to  1 L with Milli-Q/deionized water.

14.4 Filter sterilize using a 0.22µm bottle top filter.

15 On a 1.375" x 1.375" thermal label, label the bottle with the following information:

20X Potassium phosphate (KH_2PO_4 , 960mM)

130g/L Potassium phosphate, monobasic

Store at room temperature.

Sterile, 0.22µm filtered.

Date of manufacture: YYYYMMDD

10X Sodium phosphate (Na_2HPO_4 , 500mM)

15m

16 Prepare a  2 L **beaker** to mix components.

16.1 Using a  1 L **graduated cylinder**, measure  900 mL of Milli-Q/deionized water, and pour the water into the beaker.

16.2 Add a **magnetic stir bar** to the beaker and turn on the **magnetic stir plate**.



17 On a balance, measure  134 g

 Sodium phosphate dibasic heptahydrate **Santa Cruz Biotechnology** Catalog #sc-203402A

17.1 Slowly add the powder to the Milli-Q/deionized water until everything has dissolved.

17.2 Pour the solution back into a  1 L graduated cylinder.

17.3 Top up the volume to  1 L with Milli-Q/deionized water.

17.4 Filter sterilize using a 0.22µm bottle top filter.

18 On a 1.375" x 1.375" thermal label, label the bottle with the following information:

10X Sodium phosphate (Na_2HPO_4 , 440mM)

134g/L Sodium phosphate, dibasic

Store at room temperature.

Sterile, 0.22µm filtered.

Date of manufacture: YYYYMMDD

100X D-Glucose ($\text{C}_6\text{H}_{12}\text{O}_6$, 1.60M)

15m

19 Prepare a  2 L **beaker** to mix components.

19.1 Using a  1 L **graduated cylinder**, measure  700 mL of Milli-Q/deionized water, and pour the water into the beaker.

19.2 Add a **magnetic stir bar** to the beaker and turn on the **magnetic stir plate**.

20 On a balance, measure  290 g

 D-glucose **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #G7021-1KG



20.1 Slowly add the powder to the Milli-Q/deionized water until everything has dissolved.

20.2 Pour the solution back into a  1 L graduated cylinder.

20.3 Top up the volume to  1 L with Milli-Q/deionized water.

20.4 Filter sterilize using a 0.22µm bottle top filter.

21 On a 1.375" x 1.375" thermal label, label the bottle with the following information:

100X D-Glucose ($C_6H_{12}O_6$, 1.60M)

290g/L D-glucose

Store at room temperature.

Sterile, 0.22µm filtered.

Date of manufacture: YYYYMMDD

10X α-Lactose ($C_{12}H_{24}O_{12}$, 222mM)

15m

22 Prepare a  2 L **beaker** to mix components.

22.1 Using a  1 L **graduated cylinder**, measure  800 mL of Milli-Q/deionized water, and pour the water into the beaker.

22.2 Add a **magnetic stir bar** to the beaker and turn on the **magnetic stir plate**.

23 On a balance, measure  80 g

 α-Lactose monohydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L2643-500G**

23.1 Slowly add the powder to the Milli-Q/deionized water until everything has dissolved.



23.2 Pour the solution back into a  1 L graduated cylinder.

23.3 Top up the volume to  1 L with Milli-Q/deionized water.

23.4 Filter sterilize using a 0.22µm bottle top filter.

24 On a 1.375" x 1.375" thermal label, label the bottle with the following information:

10X α-Lactose ($C_{12}H_{24}O_{12}$, 222mM)

80g/L α-Lactose monohydrate

Store at room temperature.

Sterile, 0.22µm filtered.

Date of manufacture: YYYYMMDD

50% Glycerol ($C_3H_8O_3$, 6.84M, 630 g/L)

15m

25 Using a  1 L **graduated cylinder**, measure  500 mL of Milli-Q/deionized water.

26 Pour the water into a  1 L **bottle**.

27 Place the  1 L bottle on a **balance**, and zero the balance with the bottle.

28 Pour 100% glycerol into the bottle until the balance reaches  630 g .

29 Close the bottle and mix by inverting until no glycerol schlieren is visible.

30 Filter sterilize using a 0.22µm bottle top filter.

31 On a 1.375" x 1.375" thermal label, label the bottle with the following information:

50% Glycerol ($C_3H_8O_3$, 6.84M)



630 g/L Glycerol

Store at room temperature.

Sterile, 0.22µm filtered.

Date of manufacture: YYYYMMDD

1X Auto-induction Terrific Broth (made fresh from stocks per production run)

10m

32 Mix the following components in a sterile  1 L beaker. Open stock reagents under an open flame in a sterilized work area.

1.  100 mL 10X Terrific Broth without phosphates and glycerol (pour from 100 mL aliquot bottle)
2.  100 mL 10X α-Lactose
3.  100 mL 10X Sodium phosphate
4.  50 mL 20X Potassium phosphate
5.  10 mL 100X Magnesium sulfate
6.  10 mL 100X Ammonium sulfate
7.  10 mL 100X D-Glucose
8.  20 mL 50% Glycerol
9.  1 mL 1000X Antibiotic (100 mg/mL Carbenicillin, 100 mg/mL Ampicillin, 50 mg/mL Kanamycin)
10.  1 mL

 1000x Trace Metals Mixture. 1000mL, Sterile **Teknova Catalog #T1001**

33 Using a sterile  1 L **graduated cylinder**, measure  600 mL of Milli-Q/deionized water, and pour the water into the beaker to mix the components in the beaker.

34 Pour the contents of the beaker back into the  1 L **graduated cylinder**, and cover the cylinder until you are ready to use the media (within ~1 hour).



Expected result

The following describes the composition of the complete 1X Auto-induction Terrific Broth

 1 L :

24g/L Yeast extract
12g/L Tryptone
684mM Glycerol (12.6mg/L, 1.0%)
1.25mM Magnesium sulfate (0.15g/L)
25mM Ammonium sulfate (3.3g/L)
48mM Potassium phosphate (6.5g/L)
50mM Sodium phosphate (7.1g/L)
16mM Glucose (2.9g/L)
22mM Lactose (7.6g/L)

Trace metals

83.35 μM FeCl_3 (13.52mg/L)
15.73 μM MnCl_2 (1.98mg/L)
3.67 μM CoCl_2 (476 μg /L)
17.84 μM ZnSO_4 (2.88mg/L)
3.511 μM NiCl_2 (455 μg /L)
2.375 μM Na_2MoO_4 (489 μg /L)
26.49 μM CaCl_2 (2.94mg/L)
2.536 μM CuCl_2 (341 μg /L)
2.00 μM Na_2SeO_3 (346 μg /L)
2.00 μM H_3BO_3 (124 μg /L)